Urine neutrophil gelatinase-associated lipocalin is a marker of graft recovery after kidney transplantation

Maria E. Hollmen[1], Lauri E. Kyllönen[1], Kaija A. Inkinen[2], Martti L.T. Lalla[2] and Kaija T. Salmela[1]

[1]*Division of Transplantation, Department of Medicine, Helsinki University Hospital, Helsinki, Finland and* [2]*Department of Medicine, HUSLAB, Surgical Hospital, Helsinki University Hospital, Helsinki, Finland*

Delayed graft function (DGF), especially long-lasting DGF, complicates kidney transplant outcome. Neutrophil gelatinase-associated lipocalin (NGAL) is an acute kidney injury marker; therefore, we tested whether urine NGAL could predict DGF, prolonged DGF (lasting over 14 days), or the quality of kidney function in transplant recipients without DGF (non-DGF). We collected urine samples from 176 recipients transplanted with deceased donor kidneys before and various days after transplantation. A total of 70 transplantations had DGF, of which 26 were prolonged. Patients who developed DGF had a significantly slower decrease in urinary NGAL compared with those without DGF, such that day 1 NGAL predicted DGF (area under the curve (AUC) 0.75) and predicted DGF in 15 of 112 cases with day 1 urine output over 1 l (AUC 0.70) and in 19 of 86 cases with a day 1 decrease in creatinine over 50 μmol/l (AUC 0.74). The urinary NGAL level on day 1 predicted prolonged DGF (AUC 0.75), which had significantly worse 1-year graft survival (73%), compared with shorter DGF (100%). In non-DGF, high day 3 NGAL (greater than the mean) was associated with significantly worse kidney function at 3 weeks compared with low NGAL, but not at 3 months and 1 year. NGAL did not correlate with long-term function in DGF. Hence, day 1 urinary NGAL predicted DGF even when it was not clinically expected early on, and importantly, it predicted prolonged DGF that led to worse graft survival.

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Correspondence: *Maria E. Hollmen, Division of Transplantation, Department of Medicine, Helsinki University Hospital, Kasarmikatu 11-13, Helsinki 00130, Finland. E-mail: maria.hollmen@helsinki.fi*

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Delayed graft function (DGF) is an increasing problem after deceased donor kidney transplantation, as more kidneys from expanded criteria donors are accepted for transplantation. DGF is associated with acute rejection, increases the need for dialysis and posttransplantation biopsies, extends the post-transplantation hospital stay, and causes considerable economic burden.[1–8] In addition, the length of DGF is associated with worse 1-year outcome.[4,9] Ischemia-reperfusion injury occurs in all deceased donor transplantations, and has a major role in the pathogenesis of DGF. DGF can thus be regarded as one type of acute kidney injury.[10] Despite extensive studying on the mechanisms of ischemia-reperfusion injury in the experimental models, very little has been achieved in the prevention and treatment of DGF from the clinical point of view. Therefore, finding new ways to diagnose DGF very soon after, or even before transplantation, would further the possibility of developing therapeutic methods to prevent DGF in a clinical setting.

Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a new, noninvasive diagnostic tool for acute kidney injury.[11–16] NGAL associates with DGF; the association has been shown in kidney transplant biopsies taken 1 h after reperfusion, and in urine and serum samples taken on the day of, and very soon after, transplantation.[17–21]

The aim of our study was to examine (1) how serial urine NGAL (uNGAL) concentrations change over time after kidney transplantation; (2) whether uNGAL predicts the onset of kidney graft function; (3) whether uNGAL predicts prolonged DGF; and (4) whether uNGAL correlates with the level of kidney function in transplantations with early graft function (EGF).

RESULTS

The study included 176 renal transplant recipients. Recipient pretransplantation, posttransplantation, and donor characteristics are presented in Tables 1, 2, and 3. All recipients were Caucasian, except for one. The DGF grafts started to function from mean 12.0 days (s.d. 7.0) after transplantation. The donors in the DGF group were older, expanded criteria donors[22] were more common, cold ischemia time was longer, pretransplantation hemodialysis was more common, and time on dialysis before transplantation was longer in the DGF group compared with the EGF group. Overall 1-year patient

Table 1 | Recipient pretransplantation characteristics

	EGF	DGF[a]	*P*-value
N	106/176 (60.2%)	70/176 (39.8%)	
Mean age, years (s.d.)	50.5 (12.8)	54.1 (13.3)	NS
Gender			
Female	45 (42.5%)	21 (29.6%)	NS
Male	61 (57.5%)	49 (69.4%)	
TX number			
First TX	99 (93.4%)	62 (88.6%)	NS
Re-TX	7 (6.6%)	8 (11.4%)	
Underlying kidney disease			
Polycystic disease	26 (24.5%)	16 (22.9%)	NS
Glomerulonephritis	21 (19.8%)	14 (20.0%)	
Diabetes mellitus	29 (27.4%)	19 (27.1%)	
Other	30 (28.3%)	21 (30.0%)	
Mode of dialysis			
Hemodialysis	62 (58.5%)	52 (74.3%)	0.032
Peritoneal dialysis	44 (41.5%)	18 (25.7%)	
Mean time on dialysis, days (s.d.)	770 (571.1)	975 (598.9)	0.007

Abbreviations: DGF, delayed graft function; EGF, early graft function; NS, not significant; s.d., standard deviation; TX, transplantation.
[a]DGF defined according to the Halloran criteria.[23]

Table 2 | Recipient posttransplantation characteristics

	EGF (*n*=106 (60.2%))	DGF (*n*=70 (39.8%))[a]	*P*-value
Initial CNI			
Tacrolimus	24 (22.6%)	17 (24.3%)	NS
Cyclosporine A	82 (77.4%)	53 (75.7%)	NS
Induction therapy with IL-2 receptor antagonist	15 (14.2%)	13 (18.6%)	NS
Mean change in plasma creatinine from pre-TX to day 1 (μmol/l (s.d.))	−117 (145.5)	+17 (129.1)	<0.0001
Mean plasma creatinine (μmol/l (s.d.))			
Day 1	445 (198.8)	664 (191.8)	<0.0001
Day 3	250 (148.3)	644 (191.9)	<0.0001
Day 7	141 (57.2)	458 (193.9)	<0.0001
3 Weeks	120 (38.6)	206 (112.5)	<0.0001
3 Months	110 (27.4)	148 (48.8)	<0.0001
1 Year	109 (38.8)	128 (41.9)	0.002
Mean eGFR, ml/min (s.d.)			
3 Weeks	64.2 (19.9)	46.4 (21.2)	<0.001
3 Months	69.7 (23.2)	58.9 (21.1)	0.003
1 Year	74.8 (24.4)	67.7 (23.5)	0.050
Mean urine output (ml per 24 h (s.d.))			
Day 1	2544 (1526.5)	2406 (809.2)	<0.0001
Day 3	574 (613.8)	713 (713.7)	<0.0001
Day 7	2412 (779.8)	1274 (1007.5)	<0.0001
Day 14	2661 (690.6)	1888 (1060.3)	<0.0001
Number of rejections	4 (3.8%)	6 (8.6%)	NS
Mean time to rejection (days (s.d.))	8.7 (2.1)	20.8 (13.8)	NS
1-Year patient survival	99.4%	98.6%	NS
1-Year graft survival	99.1%	90.0%	0.005

Abbreviations: CNI, calcineurin inhibitor; DGF, delayed graft function; EGF, early graft function; eGFR, estimated glomerular filtration rate (Cockcroft–Gault); IL-2 receptor antagonist, interleukin-2 receptor antagonist (either basiliximab (*n*=19) or daclizumab (*n*=9)); NS, not significant; TX, transplantation; s.d., standard deviation.
[a]DGF defined according to the Halloran criteria.[23]

survival was 98.9%, and graft survival was 95.5%. The 1-year graft survival and long-term (3 month and 1 year) graft function were inferior in the DGF group, compared with the EGF group.

A pretransplantation urine sample was obtained from 70 patients, as 106 patients were anuric or oliguric before transplantation. The pretransplantation uNGAL did not correlate with residual diuresis from the native kidneys of the recipients ($R = 0.089$, $p = $ NS). The mean uNGAL levels decreased after transplantation in both DGF and EGF groups (Figure 1). The mean uNGAL concentrations were significantly lower in the EGF group compared with the DGF group at all measured time points after transplantation. Recipient's posttransplantation uNGAL was not affected by donor age, gender, or by induction immunosuppression given to the recipient (data not shown).

We included in the multivariate analysis (multilogistic regression method, forward, conditional) factors significantly differing between the DGF and EGF groups in the univariate analyses and also the clinically relevant factors in this respect, such as recipient age, donor plasma creatinine, and donor-estimated glomerular filtration rate (eGFR) (Table 4). Day 1 urine output, day 1 uNGAL, and the mode of dialysis emerged as significant, independent predictors of DGF.

Receiver operating characteristic (ROC) analyses were performed to assess the potential of uNGAL in predicting DGF. The area under the curve (AUC) for day 1 uNGAL was 0.750 (confidence interval (CI) 0.663–0.837; $P<0.0001$). At the optimal cutoff level of 560 ng/ml, the sensitivity was 68% and the specificity was 73%. The odds ratio for this cutoff level was 5.4 (CI 2.4–12.3). For comparison, day 1 urine output predicted DGF with an AUC of 0.931 (CI 0.894–0.967; $P<0.0001$). At the optimal cutoff level of 1035 ml, the sensitivity was 91% and the specificity was 80%.

The correlations between uNGAL and kidney function (plasma creatinine, eGFR) at 3 weeks, 3 months, and 1 year after transplantation were studied (Table 5). uNGAL correlated with kidney graft function up to 3 months. The mean length of stay in the hospital after kidney transplantation was 21 days (ranging from 15 to 43 days). Day 1 uNGAL did not correlate with length of stay in the hospital ($R = 0.047$, $P = $ NS).

We wanted to study whether uNGAL predicts DGF in cases in which EGF was expected on the basis of early urine output and decreasing plasma creatinine. First, we focused on the 112 transplantations with day 1 urine output >1 l. Despite good diuresis, 15 of these transplantations developed DGF. Their mean day 1 uNGAL concentration was significantly higher, 1217 ng/ml (s.d. 1228.9), compared with 460 ng/ml (s.d. 481.3, $P<0.0001$) of the 97 transplantations with EGF. Day 1 uNGAL predicted DGF in this subgroup

Table 3 | Donor characteristics

	EGF	DGF[a]	*P*-value
Mean age (years)	49.1 (9–75)	55.8 (9–75)	0.002
Gender			
Female	44 (41.5%)	28 (40.0%)	NS
Male	62 (58.5%)	42 (60.0%)	
Cause of death			
Cerebrovascular accident	75 (70.8%)	57 (81.4%)	NS
Traumatic brain injury	33 (31.2%)	13 (18.6%)	
Mean plasma creatinine (μmol/l (s.d.))	63 (21.0)	64 (17.4)	NS
Mean eGFR (ml/min (s.d.))[b]	124 (39.2)	115 (35.0)	NS
History of hypertension	26 (24.5%)	24 (34.3%)	NS
Expanded criteria donors	33 (31.1%)	36 (51.4%)	0.007
Need for cardiopulmonary resuscitation	25 (23.6%)	10 (14.3%)	NS
Antemortem intracranial surgery	19 (17.9%)	22 (31.4%)	NS
Use of inotropes	63 (59.4%)	62 (88.6%)	NS
Use of antidiuretic hormone	50 (47.2%)	38 (54.3%)	NS
Multiorgan donor	39 (36.8%)	38 (54.3%)	NS
Mean hospital days before brain death (s.d.)	2.0 (2.5)	1.6 (1.3)	NS
Mean cold ischemia time (hours (s.d.))	21.3 (3.7)	22.9 (3.6)	0.007

Abbreviations: DGF, delayed graft function; EGF, early graft function; eGFR, estimated glomerular filtration rate; NS, not significant; s.d., standard deviation.
[a]DGF defined according to the Halloran criteria.[23]
[b]eGFR estimated by the Cockcroft–Gault equation[35] in 96 adult donors and by the Schwartz equation[36] in three pediatric donors.
Expanded criteria donors were defined using the Port criteria.[22]

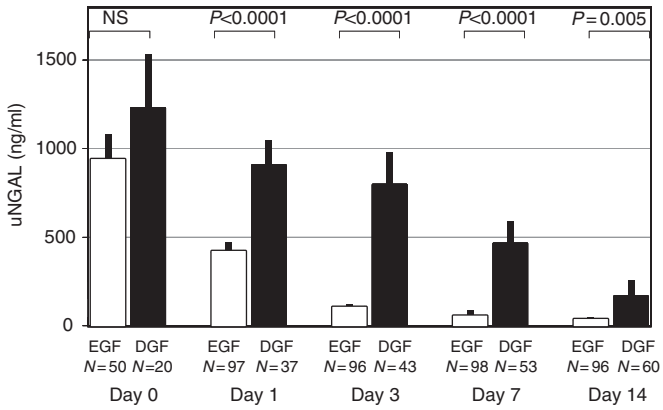

Figure 1 | The mean uNGAL concentrations between EGF and DGF groups. The mean uNGAL concentrations in DGF and EGF groups at all measured time points; before transplantation (day 0) and after transplantation (days 1, 3, 7, and 14). The results are expressed as means (+s.e.m.). DGF = delayed graft function, defined according to the Halloran criteria[23]; EGF, early graft function; NS, not significant; uNGAL, urine neutrophil gelatinase-associated lipocalin.

with an AUC of 0.696 (CI 0.526–0.866; $P=0.034$). At the 560 ng/ml cutoff level, the sensitivity was 55% and specificity was 74%. For comparison, day 1 urine output predicted DGF with an AUC of 0.803 (CI 0.705–0.902; $P<0.0001$). At the optimal cutoff level of 1690 ml, the sensitivity was 81% and the specificity was 67%.

Table 4 | Multivariate analysis of DGF[a] risk factors

	P-value
Donor age (years)	0.315
Donor plasma creatinine (μmol/l)	0.891
Donor eGFR (ml/min)	0.600
Expanded criteria donors	0.484
Cold ischemia time (hours)	0.980
Recipient age (years)	0.958
Mode of dialysis (hemodialysis or peritoneal dialysis)	0.004
Time on dialysis before TX (days)	0.457
Change in plasma creatinine from pre-TX to day 1	0.891
Recipient day 1 urine output (ml)	<0.0001
Recipient day 1 uNGAL (ng/ml)	0.019

Abbreviations: DGF, delayed graft function; eGFR, estimated glomerular filtration rate (Cockcroft–Gault); TX, transplantation; uNGAL, urine neutrophil gelatinase-associated lipocalin.
[a]DGF defined according to the Halloran criteria.[23]
Expanded criteria donors were defined using the Port criteria.[22]

Secondly, we analyzed the cases in which plasma creatinine had decreased from pretransplantation level, taken at arrival to the transplantation center, to day 1 ($n=112$). The mean decrease in plasma creatinine in these patients was 149 μmol/l (s.d. 125.2). In 86 cases, the decrease in plasma creatinine was >50 μmol/l, and 19 of these developed DGF. Their mean day 1 uNGAL concentration was significantly higher (1318 ng/ml, s.d. 1245.9) compared with that of the 67 recipients with EGF (398 ng/ml, s.d. 340.0; $P<0.0001$). A ROC analysis resulted in an AUC of 0.744 (CI 0.570–0.918; $P=0.014$). At the 560 ng/ml cutoff level, the sensitivity was 60% and specificity was 75%. For comparison, the change in plasma creatinine from pretransplantation level to day 1 could not predict DGF (AUC 0.585; $P=$ NS).

Prolonged duration of DGF

To assess uNGAL's potential in predicting prolonged DGF, we divided the 70 DGF transplantations according to the duration of DGF: ⩽7 days, 8–13 days, and ⩾14 days (Table 6, and Figure 2).

A ROC analysis for day 1 uNGAL predicted DGF lasting longer than 7 days, with an AUC of 0.748 ($P<0.0001$) (Figure 3). At the cutoff level of 560 ng/ml, the sensitivity was 70% and the specificity was 70%. Day 1 uNGAL predicted DGF lasting longer than 14 days, with an AUC of 0.748 ($P=0.005$). At the cutoff level of 560 ng/ml, the sensitivity was 83% and the specificity was 66%. We also analyzed the predictive power of day 3 uNGAL in prolonged DGF. A ROC analysis for day 3 uNGAL in predicting DGF lasting longer than 7 days produced and AUC of 0.457 (CI 0.267–0.647; $P=$ NS). A ROC analyses for day 3 uNGAL in predicting DGF lasting longer than 14 days produced an AUC of 0.608 (CI 0.422–0.793; $P=$ NS).

Conventional DGF definition and slow graft function

The DGF group had significantly higher uNGAL concentrations, compared with both slow (SGF) and immediate graft function (IGF) groups defined according to Humar *et al.*[5] (Table 7, Figure 4). Early after transplantation, the NGAL

Table 5 | The correlation between uNGAL and renal graft function at 3 months and 1 year after transplantation

	3 Weeks		3 Months		1 Year	
	Creatinine	eGFR	Creatinine	eGFR	Creatinine	eGFR
Day 1 uNGAL	R=0.382	R=0.283	R=0.189	R=0.059	R=0.010	R=0.010
	$P<0.0001$	$P<0.0001$	P=0.032	P=NS	P=NS	P=NS
Day 3 uNGAL	R=0.553	R=0.571	R=0.359	R=0.337	R=0.267	R=0.125
	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	P=0.002	P=NS
Day 7 uNGAL	R=0.499	R=0.519	R=0.242	R=0.253	R=0.152	R=0.174
	$P<0.0001$	$P<0.0001$	P=0.003	P=0.002	P=NS	P=0.035
Day 14 uNGAL	R=0.531	R=0.467	R=0.122	R=0.144	R=0.036	R=0.038
	$P<0.0001$	$P<0.0001$	P=NS	P=NS	P=NS	P=NS

Abbreviations: eGFR, estimated glomerular filtration rate (Cockcroft–Gault); NS, not significant; R, correlation coefficient; uNGAL, urine neutrophil gelatinase-associated lipocalin.

Table 6 | Transplantation characteristics by the length of DGF

	DGF ⩽7 days (n=17)[a]	DGF 8–14 days (n=27)[a]	DGF ⩾14 days (n=26)[a]	P-value between all groups
Mean donor age (years (s.d.))	55.2 (8.7)	56.0 (13.9)	56.0 (10.7)	NS
Mean cold ischemia time (hours (s.d.))	23.4 (3.3)	22.0 (3.9)	23.3 (3.4)	NS
Recipient age (years (s.d.))	54.0 (9.3)	56.2 (13.4)	52.0 (15.4)	NS
Mode of dialysis before TX				
Hemodialysis	15	21	19	NS
Peritoneal dialysis	2	6	7	NS
Mean time on dialysis before TX (days (s.d.))	1082 (675.4)	939 (694.0)	929 (430.9)	NS
Mean day 1 urine output (ml (s.d.))	755 (687.4)	519 (651.7)	439 (497.6)	NS
Mean uNGAL (ng/ml (s.d.))				
Pre-TX	1733 (1972.6)	1597 (1172.6)	1150 (536.8)	NS
Day 1	1153 (1202.1)	1044 (856.2)	963 (530.0)	NS
Day 3	774 (837.7)	982 (1211.8)	1200 (1548.1)	NS
Day 7	133 (181.5)	642 (860.2)	984 (1308.2)	0.001
Day 14	39 (29.9)	84 (89.8)	706 (1190.3)	0.004
Mean plasma creatinine at 1-year post-TX (μmol/l (s.d.))	115 (35.1)	117 (31.9)	156 (47.3)	0.002
Mean eGFR at 1-year post-TX (ml/min (s.d.))	75.5 (25.1)	70.7 (22.1)	56.4 (20.1)	0.031
1-Year graft survival	100%	100%	73.1%	0.001

Abbreviations: DGF, delayed graft function; eGFR, estimated glomerular filtration rate (Cockcroft–Gault); s.d., standard deviation; TX, transplantation; uNGAL, urine neutrophil gelatinase-associated lipocalin.
[a]DGF defined according to the Halloran criteria.[23]

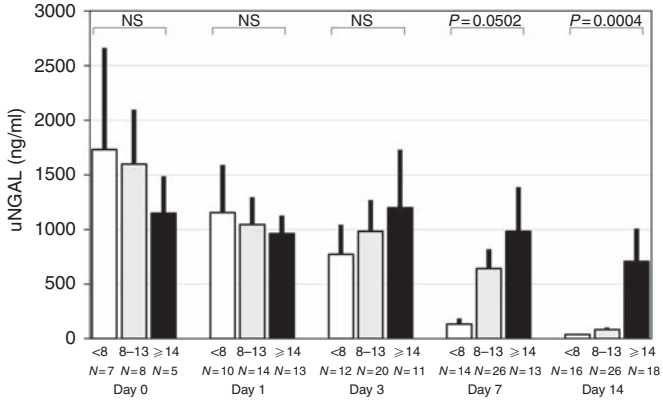

Figure 2 | The uNGAL concentrations according to the length of DGF. The mean uNGAL concentrations before (day 0) and after transplantation (days 1, 3, 7, and 14) in DGF groups with onset of function in <8 days, between 8 and 14 days, and at 14 days or later. The results are expressed as means (+ s.e.m.). DGF, delayed graft function, defined according to the Halloran criteria[23]; NS, not significant; uNGAL, urine neutrophil gelatinase-associated lipocalin.

levels in the SGF group were similar to the levels in the DGF group, and at later time points, they were similar to the IGF group. At 1 year after transplantation, the mean plasma creatinine was higher and eGFR was lower in the DGF group, compared with IGF and SGF groups.

A ROC analysis for day 1 uNGAL in predicting conventionally defined DGF produced an AUC of 0.736 (CI 0.642–0.830; $P<0.0001$). At the optimal 560 ng/ml cutoff level, the sensitivity was 65%, and the specificity was 74%. Day-1 uNGAL predicted SGF with an AUC of 0.648 (CI 0.474–0.822; P = NS).

There were no significant differences in mean uNGAL concentrations or renal transplant function between the DGF transplantations defined by the Halloran criteria,[23] and the DGF transplantations defined by the conventional definition (Table 8).

NGAL in EGF transplantations

All EGF transplantations were divided into high and low groups according to the measured uNGAL concentration on

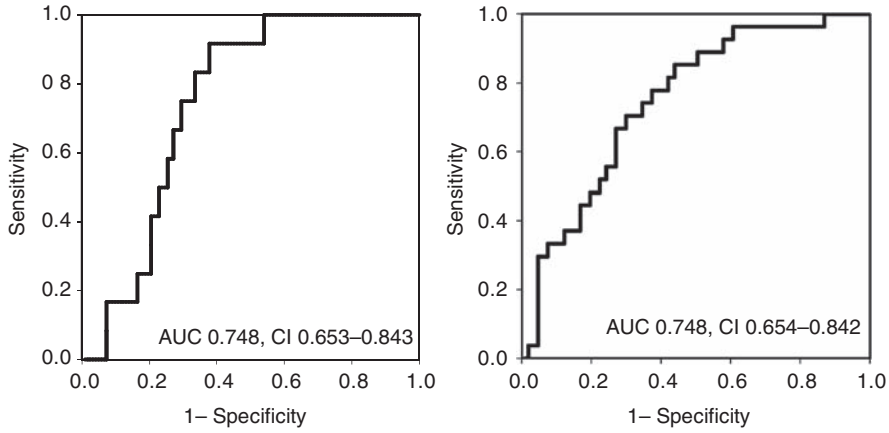

Figure 3 | The ROC curves for day 1 uNGAL in predicting DGF lasting longer than 7 days and for DGF lasting longer than 14 days. The ROC analyses for day 1 uNGAL in predicting DGF lasting longer than 7 days and in predicting DGF lasting longer than 14 days. AUC, area under the curve; CI, confidence interval; DGF, delayed graft function, defined according to the Halloran criteria[23]; ROC, receiver operating characteristic; uNGAL, urine neutrophil gelatinase-associated lipocalin.

Table 7 | Posttransplantation differences among IGF, SGF, and DGF groups

	IGF (n=94)	SGF (n=16)	DGF (n=66)	P-value between all groups
Mean uNGAL (ng/ml (s.d.))				
Pre-TX	1002 (991.9)	1858 (1775.9)	1429 (969.8)	NS
Day 1	462 (475.6)	946 (1070.8)	931 (715.1)	<0.0001
Day 3	82 (126.4)[a]	288 (163.1)[a,b]	1032 (1230.7)[b]	<0.0001
Day 7	55 (68.4)[c]	141 (189.4)[c,d]	665 (1006.1)[d]	<0.0001
Day 14	34 (110.5)	34 (27.8)[e]	274 (729.5)[e]	0.011
Mean plasma creatinine (μmol/l (s.d.))				
Day 1	425 (177.6)[a]	710 (270.0)[a]	640 (204.2)	<0.0001
Day 3	215 (94.9)[a]	653 (185.3)[a]	620 (211.7)	<0.0001
Day 7	135 (59.6)[a]	304 (136.9)[a,d]	450 (205.4)[d]	<0.0001
3 Week	116 (38.0)[b]	152 (46.0)[b]	209 (112.5)	<0.0001
3 Month	107 (25.3)[b]	128 (32.5)[b]	149 (70.6)	<0.0001
1 Year	107 (36.8)	118 (41.5)	130 (42.7)	0.002
Mean eGFR (ml/min (s.d.))				
3 Week	66.1 (19.8)	55.7 (19.0)	44.5 (19.7)	<0.0001
3 Month	71 (23.7)	66 (21.3)	57 (20.2)	0.001
1 Year	75.7 (24.5)	75.9 (26.6)	65.5 (21.5)	0.03
1-Year patient survival	98.9%	100%	98.5%	NS
1-Year graft survival	98.9%	100%	89.4%	0.012

Abbreviations: DGF, delayed graft function, defined as need for dialysis during the first week after transplantation; eGFR, estimated glomerular filtration rate (Cockcroft-Gault); IGF, immediate graft function; NS, not significant; s.d., standard deviation; SGF, slow graft function; uNGAL, urine neutrophil gelatinase-associated lipocalin.
[a]P<0.0001 between the IGF and SGF groups.
[b]P≤0.005 between the DGF and SGF groups.
[c]P=0.01 between the IGF and SGF groups.
[d]P=0.009 between the DGF and SGF groups.
[e]P=0.031 between the DGF and SGF groups.
Groups defined according to Humar *et al.*[5]

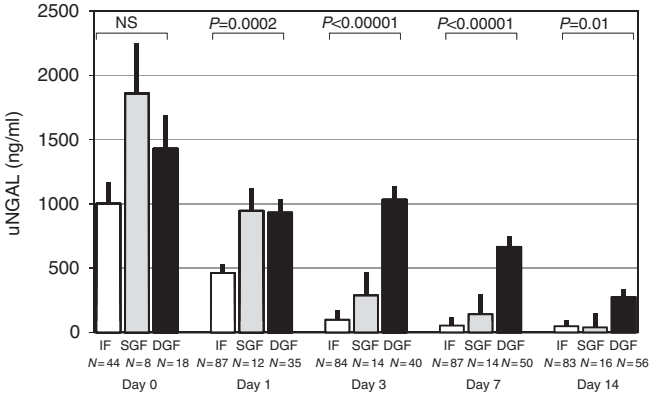

Figure 4 | The mean uNGAL concentrations in DGF, SGF, and IGF groups. The mean uNGAL concentrations in DGF, SGF, and IGF groups before transplantation (day 0) and after transplantation at all measured time points (days 1, 3, 7, and 14). Groups were defined according to Humar *et al.*[5] The results are expressed as means (+s.e.m.). DGF, delayed graft function, defined as required for dialysis during the first week after transplantation, uNGAL, urine neutrophil gelatinase-associated lipocalin; IGF, immediate graft function; SGF, slow graft function.

days 1, 3, 7, and 14, using the means 468, 120, 86, and 47 ng/ml, respectively, as cutoff points (Table 9).

High days 3 and 7 uNGAL concentrations were associated with higher plasma creatinine and lower eGFR at 3 weeks posttransplantation, but not at 3 months or 1 year after transplantation.

DISCUSSION

DGF is a common complication after kidney transplantation, and it seems to become more frequent with the relaxation of donor criteria,[24,25] as demonstrated in this report with a DGF rate of almost 40%. In the literature, the DGF rates vary in deceased donor transplantations from below 10 to 50%.[10,26] In the clinic, the diagnosis of DGF is usually made within a few days after transplantation based on diuresis, plasma creatinine, and need for dialysis. DGF is, so far, not causally treatable. Provided that the diagnosis of DGF could be made immediately after transplantation, the transplant clinicians could earlier individualize patient care, for example, by

adjusting the initiation and dose of calcineurin inhibitors, avoiding other nephrotoxic agents, and scheduling for posttransplantation dialysis. In addition, this would enable the development of new DGF treatment strategies in the future.

In kidney transplantation, the inevitable cold ischemia and warm reperfusion affect the distal nephron and result in similar morphological and functional findings, as found in human acute kidney injury in general.[27] In rodents, the main site for NGAL production is the distal nephron.[28] Kidney transplantation can be regarded as a model for human acute kidney injury in which NGAL production occurs.[27] NGAL has also been suggested as an early marker for DGF,[17–21] but the data on that are still scarce. The similarities, differences, and limitations regarding previous reports on NGAL and clinical kidney transplantation[17–21] are listed in Table 10. In this study, we wanted to test the findings of the few previous studies in a larger kidney transplant patient population, with a special emphasis on prolonged DGF. Prolonged DGF has been found to be more detrimental to the transplanted kidney, compared with DGF of a few days' duration,[4,9] as also demonstrated in this study. We were also interested to see whether uNGAL has any importance in renal transplants with EGF.

We chose urine as sample material, as uNGAL has been suggested to represent the damage in the kidney,[29] and it has previously been shown to result from local synthesis in the kidney rather than filtration from blood.[30]

The uNGAL levels have previously been shown to be elevated in patients with chronic kidney disease.[31,32] In this study, the pretransplantation uNGAL is representative of levels in dialysis-dependent patients with end-stage renal disease. The high uNGAL levels in these patients might just result from decreased GFR, but also might reflect ongoing damage in the kidney.[33] We used the pretransplantation concentrations as a reference for posttransplantation uNGAL measurements. uNGAL levels were similar in EGF and DGF groups, and were not used in predicting onset of function after transplantation.

In this study, the donors were older, expanded criteria donors were more common, mean cold ischemia time was longer, pretransplantation hemodialysis was more common,

Table 8 | The differences between DGF groups defined by the Halloran criteria[23] and the conventional criteria (need for posttransplantation dialysis during the first week after transplantation)

	DGF Halloran (*n*=70)	DGF conventional (*n*=66)	*P*-value
Mean day 1 urine output (ml (s.d.))	574 (611.8)	497 (536.0)	NS
Mean uNGAL (ng/ml (s.d.))			
Pre-TX	1533 (1360.6)	1429 (969.8)	NS
Day 1	1045 (851.7)	931 (715.1)	NS
Day 3	979 (1201.5)	1032 (1230.7)	NS
Day 7	591 (924.8)	665 (1006.1)	NS
Day 14	259 (706.8)	274 (729.5)	NS
Mean plasma creatinine (μmol/l (s.d.))			
Day 1	662 (209.9)	640 (204.2)	NS
Day 3	644 (205.3)	620 (211.7)	NS
Day 7	458 (198.9)	450 (205.4)	NS
3 Week	206 (111.5)	209 (112.5)	NS
3 Month	147 (69.1)	149 (70.6)	NS
1 Year	128 (41.5)	130 (42.7)	NS
Mean eGFR at 1-year post-TX (ml/min (s.d.))	67.7 (23.4)	65.5 (21.5)	NS
1-Year graft survival	90.0%	89.4%	NS

Abbreviations: DGF, delayed graft function; eGFR, estimated glomerular filtration rate (Cockcroft-Gault); NS, not significant; s.d., standard deviation; uNGAL, urine neutrophil gelatinase-associated lipocalin.

Table 9 | High (above the mean) and low (below the mean) uNGAL concentration and kidney transplant function at 3 weeks, 3 months, and 1 year after transplantation in the transplantations with EGF

	3 Weeks			3 Months				1 Year			
	eGFR		Creatinine		eGFR		Creatinine		eGFR		Creatinine	
Day 1 uNGAL												
High (⩾468 ng/ml)	65 (19.1)	NS	126 (44.8)	NS	71 (25.0)	NS	112 (28.4)	NS	80 (27.9)	NS	107 (28.9)	NS
Low (<468 ng/ml)	66 (19.9)		114 (29.8)		70 (21.9)		105 (24.8)		75 (22.5)		109 (43.7)	
Day 3 uNGAL												
High (⩾120 ng/ml)	52 (13.9)	0.001	150 (40.3)	<0.0001	68 (25.0)	NS	116 (21.5)	0.027	74 (27.1)	NS	118 (48.0)	NS
Low (<120 ng/ml)	68 (20.3)		110 (31.4)		72 (23.1)		104 (27.1)		77 (23.4)		105 (36.0)	
Day 7 uNGAL												
High (⩾86 ng/ml)	51 (12.2)	<0.0001	146 (43.9)	0.001	67 (26.0)	NS	113 (19.8)	NS	74 (30.9)	NS	115 (49.3)	NS
Low (<86 ng/ml)	68 (19.5)		114 (35.4)		72 (22.8)		108 (28.3)		75 (23.4)		109 (39.9)	
Day 14 uNGAL												
High (⩾47 ng/ml)	56 (19.0)	0.015	127 (32.4)	NS	67 (18.2)	NS	102 (16.0)	NS	76 (18.8)	NS	94 (26.7)	NS
Low (<47 ng/ml)	66 (19.4)		118 (37.4)		69 (24.8)		110 (29.6)		73 (24.9)		113 (41.1)	

Abbreviations: EGF, early graft function; eGFR, estimated glomerular filtration rate (Cockcroft-Gault); NS, not significant; uNGAL, urine neutrophil gelatinase-associated lipocalin.
The data are expressed as means with standard deviation.

Table 10 | Comparison between previous studies on NGAL and prediction of DGF and our study

First author	Similarities	Differences	Limitations
Mishra *et al.*[17]	Single center Prospective Single biomarker	Sample material (biopsies) Method (IHC) Patient population	Solely pediatric patients Number of patients limited (*n*=25) Living and deceased donor TXs No analyses of other factors associated with DGF (age, CIT, and so on) No multivariate analysis
Parikh *et al.*[18]	Sample material (urine) Single center Prospective	Method (ELISA) Sample collection and timing Patient population Multibiomarker	Both adult and pediatric patients Living and deceased donor TXs No standardized sampling No data on anuric patients/patients without sample Number of patients limited (*n*=53) No data on pretransplantation NGAL
Kusaka *et al.*[20]	Single center Prospective Single biomarker	Sample material (serum) Method (ELISA) Patient population	Living and non-heart beating donor TXs Number of patients limited (*n*=16) No analyses of other factors associated with DGF (age, CIT, and so on) No multivariate analysis
Lebkowska *et al.*[21]	Sample collection time points Single center Prospective Single biomarker	Sample material (serum) Method (ELISA) Solely hemodialysis patients	Number of patients limited (*n*=41) DGF not defined No analyses of other factors associated with DGF (age, CIT, and so on) No multivariate analysis
Hall *et al.*[19]	Patient population Solely deceased donor TXs Sample material (urine) Prospective	Multicentre Method (ELISA) Standardized sampling during the first postoperative day Multibiomarker	No data on anuric patients/patients without sample No long-term follow-up No data on pretransplantation NGAL

Abbreviations: CIT, cold ischemia time; DGF, delayed graft function; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; TX, transplantation.

and time on dialysis before transplantation was longer in the DGF group, compared with the EGF group. All these are regarded as significant risk factors for DGF.[34] However, in our multivariate analysis, only day 1 uNGAL, day 1 urine output, and mode of dialysis emerged as independent predictors for DGF. As our ROC analysis showed, day 1 uNGAL predicted DGF, but moderately compared with day 1 urine output in this study. The AUC was also inferior compared with those reported previously.[18,19] Parikh *et al.*[18] reported an excellent AUC for uNGAL in prediction for DGF, but the patient population was smaller and more heterogeneous compared with our study. Hall *et al.*[19] reported a slightly better AUC for uNGAL in prediction of DGF compared with ours, but inferior compared with the Parikh *et al.* study. The patient population in the Hall study is similar to our study, apart from being a multicenter study. The different NGAL analysis methods and sample materials maybe other reasons why our results are not as optimistic as those reported previously (Table 10).

In clinical practice, the early diagnosis of DGF is most difficult in cases in which diuresis or decreasing plasma creatinine seem to suggest EGF, but the patient, nevertheless, develops DGF. In these cases, day 1 uNGAL gave additional value in the prediction of DGF at a time when a clinical diagnosis of DGF was yet impossible. Of great importance is our finding that day 1 uNGAL predicted prolonged DGF, which is a clinically significant complication, leading to significantly worse 1-year graft survival compared with short DGF.

We used Halloran's DGF definition,[23] as it takes into account all clinically relevant indicators of poor allograft function. This definition includes patients with good diuresis, but without decreasing plasma creatinine, and excludes patients needing only one dialysis session after transplantation because of, for example, high potassium or fluid overload. However, the most commonly used definition for DGF is need for dialysis during the first week after transplantation. Lack of uniform DGF definition complicates comparison of research data. Thus, we found it important to test the predictive value of uNGAL also with the commonly used DGF definition, and found no significant differences in our results when either of these definitions was used.

All the transplanted grafts, and also the EGF grafts, are susceptible to ischemia-reperfusion injury and the damage caused by it. Therefore, we examined whether uNGAL could predict the level of graft function in EGF transplantations. High uNGAL levels were associated with worse kidney function at 3 weeks posttransplantation, but not at 3 months, or at 1 year after transplantation. This may suggest that high uNGAL in the EGF transplantations reflects a more pronounced short-term injury, which seems to be repairable and is not seen in the long run. Furthermore, the uNGAL concentrations in the EGF transplantations decreased within 3 days nearly to the levels seen in healthy individuals. This also enables the use of uNGAL as a marker for new-onset kidney injury in kidney recipient follow-up.

There are certain limitations to this study. It was a single-center study. As we examined only one biomarker, we could

not evaluate our NGAL results in relation to other potential DGF biomarkers. We learnt from this study that the urine samples taken on the first day after transplantation are the most important; thus, the sample collection should have been better standardized during the early hours after transplantation. Confounding effects of medication, surgery, dialysis, and urine composition on NGAL analyzes, are not known and could not be eliminated.

Our study also has several strengths. It was a nationwide study, with a background population of 5 million people, and it is so far the largest study on NGAL and DGF. We analyzed our data using two different DGF definitions. We analyzed pretransplantation uNGAL levels to evaluate uNGAL concentration in dialysis-dependent patients with end-stage kidney disease. After transplantation, uNGAL was measured at several time points, and we are the first to report uNGAL levels in kidney transplant patients during the first 2 weeks after transplantation and the association of uNGAL and 1-year graft function. We also studied uNGAL levels in cases in which EGF was expected on the basis of diuresis and creatinine decrease, as DGF diagnosis is the most difficult in this patient group.

On the basis of these results, we suggest the following clinical implications (1) uNGAL allows prediction of DGF, even in patients with good urine output and decreasing creatinine; (2) low uNGAL levels in oliguric patients after kidney transplantation suggest that oliguria might also be caused by other reasons, such as suboptimal fluid balance; (3) uNGAL predicts prolonged DGF and thus identifies patients with severe kidney injury and inferior long-term outcome; (4). uNGAL provides a simple test to quantify the recovery from kidney injury. If uNGAL reaches normal values, a subsequent rise in uNGAL concentration would be suggestive of a new kidney injury. In the future, studies are needed to evaluate the association of NGAL and kidney histology during DGF, and the role of NGAL in other situations of kidney transplant dysfunction such as rejection, calcineurin toxicity, and *de novo* glomerulonephritis. In the most optimal scenario, NGAL measured in the donor before organ retrieval, could predict DGF.

In conclusion, day 1 uNGAL predicted DGF with moderate sensitivity and specificity in cases in which EGF was, according to early clinical findings, expected but ultimately did not occur. uNGAL predicted prolonged DGF on the first day after transplantation. The definition of DGF did not affect our results. In the EGF transplantations, high uNGAL was associated with worse level of kidney function during the early weeks after transplantation, but did not have long-term effects.

PATIENTS AND METHODS

The study population consisted of 176 adult, dialysis-dependent, kidney transplant recipients, recruited between August 2007 and December 2008, and their 99 consecutive, deceased donors in the Helsinki University Hospital, the only transplantation center in Finland. The minimum follow-up was 1 year. A written informed consent was obtained from the recipients before enrollment. The study protocol was approved by the local ethics committee.

The clinical data were obtained from the patients' records and the Finnish Kidney Transplant Registry database. Donor variables collected were age, gender, history of hypertension, plasma creatinine, eGFR estimated by the Cockcroft–Gault equation[35] in adults, and by the Schwartz equation[36] in three pediatric donors, need for antemortem intracranial surgery, need for cardiopulmonary resuscitation, use of inotropes and antidiuretic hormone, length of hospital stay before brain death, cause of death, expanded criteria donors, multiorgan or kidney-only donation, and cold ischemia time. Expanded criteria donors were defined as donors over 60, or over 50 years of age, with at least two of the following criteria: hypertension, plasma creatinine $>132\,\mu mol/l$, and cerebrovascular accident as cause of death.[22] None of the donors had diabetes, and all were Caucasian. All donors received intravenous steroids before the organ retrieval operation and Mannitol before *in situ* perfusion. University of Wisconsin solution was used for *in situ* perfusion and cold storage preservation of the kidneys.

Recipient variables collected were age; gender; underlying kidney disease; number of previous transplants; mode of and time on dialysis before transplantation; urine output daily after transplantation during the stay in the Transplant unit; plasma creatinine on arrival for transplantation, daily after transplantation during the stay in the transplant unit, and at 1 year; and eGFR at 3 weeks, 3 months, and 1 year after transplantation. Calcineurin inhibitor (cyclosporine ($n=135$) or tacrolimus ($n=41$)) was administered orally to all recipients before transplantation, and continued after transplantation, with target levels of 200–250 μg/ml for cyclosporine and 6–12 μg/ml for tacrolimus. Other immunosuppression consisted of mycophenolate mofetil (*target dose*: 1 g twice a day for patients with cyclosporine, and 500 mg twice a day for patients with tacrolimus) and steroids. Induction immunosuppression with interleukin-2 receptor antagonist was given to 28 recipients (basiliximab ($n=19$) and daclizumab ($n=9$)).

The primary outcome variable was DGF. DGF was defined, as described by Halloran *et al.*,[23] as oliguria $<1\,l$ every 24 h for >2 days, or plasma creatinine concentration $>500\,\mu mol/l$ throughout the first week, or >1 dialysis session needed during the first week. The results were also calculated using the conventional DGF definition: need for dialysis during the first week after transplantation. Here, the early functioning grafts were additionally divided into two groups, according to Humar *et al.*,[5] to SGF, in which plasma creatinine remained $>265\,\mu mol/l$ for >5 days after transplantation without need for dialysis, and to IGF.

NGAL sample collection and detection

Urine samples for NGAL assays were taken on arrival to the transplant unit (pretransplant sample), and after transplantation in the morning of days 1, 3, 7, and 14. The

pretransplantation sample was taken to evaluate uNGAL concentration in dialysis-dependent patients with end-stage kidney disease, and used as a reference level for uNGAL measurements after transplantation. Before transplantation, 49 patients were anuric and 57 patients were oliguric (urine output <500 ml every 24 h). The pretransplantation urine sample was obtained from 70 recipients. Day-1 urine samples were taken in the first morning after the transplant surgery in the ward, at a mean of 12.2 h (range 2.1–31.3) after reperfusion of the graft, from the urine catheter. The urine samples at later time points were collected from the urine catheter or using the clean-catch method. The day 1 urine sample was obtained from 134 patients, the day 3 urine sample from 139 patients, the day 7 urine sample from 151 patients, and the day 14 sample from 156 patients. After collection, the urine samples were immediately centrifuged, and the supernatant was stored at $-70\,^{\circ}C$ without delay.

The uNGAL assay was performed by a two-step chemiluminescent microparticle immunoassay on a standardized clinical platform (ARCHITECT, Abbott Diagnostics, Abbott Park, IL, USA), as previously described.[37]

Statistical analyses

SPSS software, version 16.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Data are expressed as means with standard deviations (s.d.) or as absolute numbers with percentages. All analyzed variables were tested for distribution. The *t*-test and analysis of variance were used for samples with normal distribution, and Mann–Whitney *U*- and Kruskal–Wallis tests were used for samples with skewed distribution. χ^2- and Fisher's exact tests were used in the analyses of contingency tables. The correlations were analyzed using the Pearson's correlation coefficient for normally distributed parameters and the Spearman correlation coefficient for parameters with skewed distribution. To assess DGF predictors, a multilogistic regression analysis (forward, conditional) was used. Factors significantly differing between the DGF and EGF groups in the univariate analyses (donor age, number of expanded criteria donors, cold ischemia time, mode of and time on dialysis before transplantation, mean change in creatinine from pretransplantation to day 1, day 1 urine output, and day 1 uNGAL), and also the clinically relevant factors in this respect (donor plasma creatinine, donor eGFR, and recipient age) were included in the multivariate analysis. The factors in the multivariate analysis consisted of categorical variables (mode of dialysis, expanded criteria donors) and the covariates consisted of continuous variables. ROC analyses were performed to assess NGAL's potential to predict DGF. The optimal cutoff level was defined by the largest sum of sensitivity and specificity. The odds ratio was calculated for the optimal AUC. A *P*-value <0.05 was considered significant.

DISCLOSURE

All the authors declared no competing interests.

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